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CHAKKARAKOLLI (*GYMNEMA SYLVESTRE*) EXTRACTS IN THE STABILIZATION OF *ALOE VERA* GEL

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ABSTRACT

An experiment was conducted in the Dept. of Plantation Crops and Spices, College of Agriculture, Vellayani, Kerala Agricultural University, during 2016 -17 for the development of a protocol for the stabilization of fresh aloe gel using chakkarakolli (Gymnema sylvestre) extracts. Fresh aloe gel samples were treated with three forms of chakkarakolli extracts, in order to find out their efficiency in the stabilization of aloe gel. Preliminary observations on pH and conductivity for a period of one week revealed the efficiency of certain treatments in maintaining a stable pH and conductivity, and the TSS and Vit C content of such promising treatments with agreeable visual quality and odour were analyzed for a period of one month, and were compared with fresh aloe gel and also with aloe gel treated with a chemical preservative (sodium benzoate) as control. 5ml and 3ml chakkarakolli alcoholic extract treated samples recorded significantly superior values compared to control, up to two weeks of storage, which declined later. After two weeks of storage, assessment of microbial load of the samples revealed no bacterial, fungal, actinomycetes and E.coli colonies in aloe gel samples treated with 5ml alcoholic extract of chakkarakolli.

KEYWORDS: Chakkarakolli, Stabilization & Aloe Gel

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INTRODUCTION

Today, the *Aloe vera* industry is flourishing and the gel is used in many products such as fresh gel, juice and other formulations for health, medicinal and cosmetic purpose (Eshun and He, 2004). However, the fast expanding *Aloe vera* industry urgently needs reliable testing protocols to assess the quality and quantity of bioactive chemicals present in the final products (Bozzi *et al*. 2007). Looking to the importance of biologically active components possessed by the leaves of the *Aloe vera* plant and its wide spread use, it has become imperative that, the leaf should be processed with the aim of essential bioactive components (Chandegara and Varshney, 2013). Fresh aloe gel degrades very rapidly at room temperature or when exposed to air. Decomposition of the gel matrix starts just after harvesting due to the action of enzymes and of bacteria normally present in leaves, resulting in loss of biological activity (Ramachandra and Rao, 2008). In order to preserve its components and to ensure the quality, it must be processed as soon as possible after cutting, through a process called “stabilization”. Unfortunately, because of improper processing procedure aloe products contain very little or virtually no active ingredients (Ramachandra and Rao, 2009). So, it has become very important to evolve a better method of preservation for increasing the shelf life and maintaining the quality of *Aloe vera* gel. Parameters that are routinely

used in the evaluation and identification of commercial *Aloe vera* gels are pH, malic acid, and conductivity (Ni and Tizard, 2004). The International Aloe Science Council (IASC) has presented guidelines for levels of these parameters in *Aloe vera* gels (Waller *et al.,* 2004). Reports regarding the synergistic effects of four species of plants belonging to Liliaceae, Zingiberaceae, Theaceae and Punicaceae on stabilization of aloe gel and their potential as natural antiseptics and oxidation resistant materials are available (JianGuo *et al.,* 2004). The efficiency of *Gymnema sylvestre* (Family: Asclepediaceae), a medicinal herb with reported antioxidant potential, in the stabilization of aloe gel has been explored in the present study.

MATERIALS AND METHODS

The experiment was conducted in the Dept. of Plantation Crops and Spices, College of Agriculture, Vellayani, Kerala Agricultural University, during 2016 -17 for the development of a protocol for the stabilization of fresh aloe gel using *Gymnema sylvestre* extracts . Fresh aloe gel samples were treated with three forms (aqueous, decoction, and alcoholic) of *Gymnema sylvestre* extracts. Aqueous extract was prepared by grinding 10g of fresh herb with 50ml of distilled water using mortar and pestle followed by filtration. Hot water extract was prepared by boiling 10g of fresh herb in 50ml of water followed by filtration, while alcoholic extract was prepared by grinding 10g of sample with 50ml of 80 % ethanol, followed by filtration. For standardizing the dosage, 20 ml of liquidized and filtered *Aloe vera* gel was treated with graded volumes (1.0ml, 2.0ml, 3.0ml, 4.0ml and 5.0ml) of each form of extract with three replications and kept at ambient temperature. Observations on pH and conductivity of each sample were recorded at weekly intervals, for a period of one month and compared with fresh aloe juice and also with aloe juice treated with a chemical preservative (sodium benzoate) as control. pH was measured using pH meter (Digital pH meter MK VI) and conductivity using Elico Digital Conductivity meter (Model CM 180). Samples which maintained a stable pH and conductivity and with agreeable visual quality and odour, were subjected to nutrient analysis. TSS and Vit C content of such promising treatments were analysed for a period of one month. For measuring, T.S.S Hanna Refractometer (Model HI 96801) was used while Vit C content was estimated following the procedure recommended by Sadasivam and Manickam, 2008. Microbial load of these samples were also estimated after two weeks of storage by the serial dilution plate technique (Johnson and Curl, 1972).

RESULTS AND DISCUSSIONS

Variation in pH in Chakkarakolli Treated Samples

Observations on the pH of treated samples during the storage period are given in Table1. On the first day of observation, no significant difference in pH was noticed among the samples and all values agreed with the values prescribed by IASC (4.5 – 4.9). One week after storage slight change in pH was observed in all samples except 1ml, 5ml hot water extract treated samples (T1,T5), 1ml, 3ml, 4ml aqueous extract (T6, T8, T9), alcoholic extract (T11 to T15) and chemical preservative (T16) treated samples. No variation in pH was noticed throughout the observational period in alcoholic extract treated samples (T12, T13, T14, T15 and T16).

Table 1: Variation in pH in Chakkarakolli Treated Samples at Weekly Intervals

Treatments	0^{th} day	7^{th} day	14^{th} day	21^{st} day	28^{th} day
T1 - 20ml gel + 1ml h.w.e.	4.7	4.7	4.6	5.3	6.6
T2 - 20ml gel + 2ml h.w.e.	4.7	4.6	4.5	5.2	6
T3 - 20ml gel + 3ml h.w. e.	4.7	4.6	4.5	5.2	6
T4 - 20ml gel + 4ml h.w.e.	4.7	4.6	4.5	5	5.7
T5 - 20ml gel + 5ml h.w.e.	4.6	4.6	4.4	5	5.4
T6 - 20ml gel + 1ml aq.e.	4.6	4.6	4.6	5.4	6.4
T7 - 20ml gel + 2ml aq.e.	4.7	4.6	4.5	5.3	6.1

Table 1: Contd.,					
T8 - 20ml gel + 3ml aq.e.	4.6	4.6	4.5	5.3	5.8
T9 - 20ml gel + 4ml aq.e.	4.6	4.6	4.5	5.1	5.7
T10 - 20ml gel + 5ml aq.e.	4.6	4.5	4.4	5	5.3
T11 - 20ml gel + 1ml al.e.	4.7	4.7	4.7	4.8	4.8
T12 - 20ml gel + 2ml al. e.	4.8	4.8	4.7	4.8	4.8
T13 - 20ml gel + 3ml al.e.	4.8	4.8	4.8	4.8	4.8
T14 - 20ml gel + 4ml al.e.	4.8	4.8	4.8	4.8	4.8
T15 - 20ml gel + 5ml al.e.	4.8	4.8	4.8	4.8	4.8
T16 - 20ml gel + c.p.	4.8	4.8	4.8	4.8	4.8
T17 – control	4.8	5	5.3	5.8	6.4
CD	0.02	0.02	0.2	0.01	0.02
SE (+-)	0.011	0.011	0.011	0.005	0.011

h. w. e – hot water extract **aq. e.** - aqueous extract **al. e.** – alcoholic extract

c. p. – chemical preservative.

Lodi and Rossin, (1995) reported wide variation in pH (4.8 - 6.4) of fresh juice during storage. According to them, the conversion of malic acid into lactic acid during storage lowers the pH of fruit juices. In the case of aloe gel, malic acid is an excellent indicator of gel freshness. This acid is produced naturally in aloe leaves during Crassulacean Acid Metabolism (CAM). Under poor handling/storage conditions, in the presence of bacteria, malic acid can be broken down to form lactic acid, which might have resulted in the increase in pH of stored gel. Thus, lactic acid occurrence is an indication of gel decay (O'Brien et al., 2011). In the present study, addition of alcoholic extracts of *Gymnema sylvestre* to the gel samples might have prevented the gel decay, thereby maintaining a steady pH throughout the storage period.

Variation in Conductivity in Chakkarakolli Treated Samples

In the case of conductivity, also similar trend was noticed. On the first day of observation no significant difference in conductivity among the samples were noticed and all maintained a stable value (2.6 – 2.7 mS). One week after storage, a slight increase in conductivity was noticed in all samples except 2ml hot water extract (T2), 1ml, 2ml, 3ml aqueous extract (T6, T7, T8) and 1ml, 3ml, 4ml, 5ml alcoholic extracts (T11, T13, T14, T15). The alcoholic extracts (T11, T13, T14, T15) treated samples and chemical preservative (T16) maintained a steady conductivity of 2.6 throughout the observational period. The fresh juice shows wide variation from initial value 2.8 to 3.0 (Table 2) during storage at room temperature.

Table 2: Variation in Conductivity in Chakkarakolli Treated Samples at Weekly Intervals (in mS)

Treatments	0th day	7th day	14th day	21st day	28th day
T1 - 20ml gel + 1ml h.w.e.	2.7	2.8	2.8	2.8	2.9
T2 - 20ml gel + 2ml h.w.e.	2.7	2.7	2.8	2.8	2.9
T3 - 20ml gel + 3ml h.w. e.	2.6	2.7	2.8	2.8	2.9
T4 - 20ml gel + 4ml h.w.e.	2.6	2.7	2.7	2.8	2.8
T5 - 20ml gel + 5ml h.w.e.	2.6	2.7	2.7	2.8	2.8
T6 - 20ml gel + 1ml aq.e.	2.7	2.7	2.8	2.8	3.0
T7 - 20ml gel + 2ml aq.e.	2.7	2.7	2.8	2.8	2.9
T8 - 20ml gel + 3ml aq.e.	2.7	2.7	2.8	2.8	2.9
T9 - 20ml gel + 4ml aq.e.	2.6	2.7	2.8	2.7	2.8
T10- 20ml gel + 5ml aq.e.	2.6	2.7	2.8	2.7	2.8
T11 - 20ml gel + 1ml al.e.	2.6	2.6	2.6	2.6	2.6
T12- 20ml gel + 2ml al. e.	2.7	2.6	2.7	2.7	2.6
T13 - 20ml gel + 3ml al.e.	2.6	2.6	2.6	2.6	2.6
T14 - 20ml gel + 4ml al.e.	2.6	2.6	2.6	2.6	2.6
T15 - 20ml gel + 5ml al.e.	2.6	2.6	2.6	2.6	2.6

Table 2: Contd.,					
T16 - 20 ml gel + c.p.	2.6	2.7	2.6	2.6	2.6
T17 – control	2.6	2.7	2.8	2.9	3.0
CD	0.02	0.01	0.02	0.01	0.02
SE (+-)	0.011	0.005	0.011	0.005	0.011

h. w. e – hot water extract **aq. e.** - aqueous extract **al. e.** – alcoholic extract

c. p. – chemical preservative

Older gels show an increase in conductivity. A possible explanation for this relationship is that, glucose can also be converted into lactic acid which can result in an increase free ions or conductivity within decaying aloe gels. Levels of conductivity in gels appear to be species specific (O'Brien *et al*., 2011). Here also, initially no significant difference in conductivity among the samples was noticed, while the values changed with treatments during storage. Maintenance of a steady conductivity in T11, T13, T14, T15 indicate that addition of *Gymnema sylvestre* alcohol extract could prevent gel decay during storage at room temperature.

During storage of aloe gel, changes in pH levels and conductivity occur as a result of the change in acid composition and concentration. Acid composition, concentration, pH and conductivity all play an important role in the colour, taste and stability of fruit juices and must be considered as a quality guide line in aloe gels (O'Brien *et al*., 2011).

Variation in TSS and Vitamin C Concentration in Treated Samples

After one week of preliminary observation on pH and conductivity, treatments without much variation in pH and conductivity during storage and with agreeable visual quality and odour were analyzed for their TSS and vit C content. These included T5 (5ml hot water extract), T10 (5ml aqueous extract), T11, T13 and T15 (1ml, 3ml and 5 ml alcoholic extracts) which were compared with T16 (fresh juice) and T17 (with chemical preservative) (Table 3).

After one week of storage at room temperature, the treatments showed wide variation in TSS (12.00– 20.60) and T16 recorded the highest value (20.60) which was on par with T15. A general reduction in TSS was noticed during storage, and during the 2nd week also T16 recorded the highest value. However, T15 also maintained a comparable value (19.40) with T16. In the case of control, a drastic reduction in TSS was noticed throughout the storage period (Table 3).

Table 3: Variation in TSS in *Gymnema Sylvestre* Treated Samples (in mg/dL)

Treatments	07th day	14th day	21st day	28th day
T5 - 20ml gel + 5ml h.w.e.	12.60	11.80	11.20	10.60
T10 - 20ml gel + 5ml aq.e.	12.20	11.40	11.00	10.20
T11 - 20ml gel + 1ml al.e.	16.00	16.20	15.80	12.60
T13 - 20ml gel + 3ml al.e.	18.60	17.80	16.60	13.00
T15- 20ml gel + 5ml al.e.	19.80	19.40	17.40	13.80
T16 - 20ml gel + c.p.	20.60	19.80	18.20	13.50
T17 – control	12.00	11.40	10.80	10.10
CD	0.91	1.40	0.91	0.78
SE (+-)	0.540	0.831	0.540	0.463

h. w. e – hot water extract **aq. e.** - aqueous extract **al. e.** – alcoholic extract

c. p. – chemical preservative

In the case of Vit C also wide variation was noticed among treatments (34.04 – 48.93) one week after storage. T15 recorded the highest value (48.93) which was on par with T13 (44.65). A general reduction in Vit C content was noticed during storage; however, during the 2nd week also T15 recorded the highest value (46.85) which was comparable with that of fresh gel. In the case of control, a drastic reduction in Vit C was noticed throughout the storage period (Table 4).

Table 4: Variation in Vitamin C Concentration in *Gymnema Sylvestre* Treated Samples (in mg/dL)

Treatments	07th day	14th day	21st day	28th day
T5 - 20ml gel + 5ml h.w.e.	34.04	27.66	25.53	23.16
T10 - 20ml gel + 5ml aq.e.	36.17	34.04	29.78	27.66
T11 - 20ml gel + 1ml al.e.	42.50	40.40	38.29	36.17
T13 - 20ml gel + 3ml al.e.	44.65	44.65	42.50	40.40
T15 - 20ml gel + 5ml al.e.	48.93	46.85	44.65	42.65
T16 - 20ml gel + c.p.	42.50	40.40	38.29	36.17
T17 – control	36.17	31.91	29.78	25.53
CD	5.39	6.00	2.71	6.14
SE (+-)	3.200	3.562	1.609	3.646

h. w. e – hot water extract **aq. e.** - aqueous extract **al. e.** – alcoholic extract

c. p. – chemical preservative

Appreciable TSS and vitamin C content was recorded by alcoholic extracts treated samples. Hence, using alcoholic extracts of *Gymnema sylvestre* aloe gel can be preserved up to two weeks without much loss in quality. Increases in pH values are expected, as lactic acid is a weaker acid than malic acid. So, this may cause increased value in pH and conductivity and decreased sugar concentration in decayed samples (Tungala *et al*., 2011).

Microbial Load in Treated Samples

Assessment of microbial load of the samples revealed no bacterial, fungal, actinomycetes and E.Coli colonies in the samples treated with *Gymnema sylvestre* alcoholic extract (5.0ml) during this period. In all other samples, varying levels of microbial population was seen.

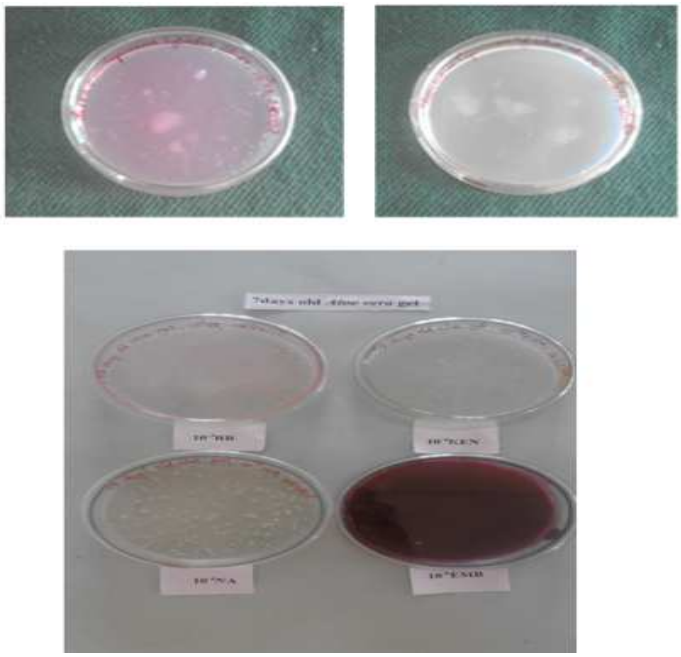

Figure 1: Microbial Load of 5ml Alcoholic Extract of *Gymnema Sylvestre* Treated Samples (after Two Weeks) and Fresh Gel (after One Week) of Storage

Plants, rich in tannins have antibacterial potential due to their basic character that allows them to react with proteins to form stable water soluble compounds, thereby killing the bacteria by directly damaging its cell membrane (Elmarie and Johan, 2001). Flavonoids are a major group of phenolic compounds reported for their antiviral (Chiang *et al*.,

2003), antimicrobial (Maria *et al*., 2009) and spasmolytic properties (Amor *et al*., 2005). Alkaloids isolated from plant are commonly found to have antimicrobial properties (Ahmedel *et al*., 2010). The antimicrobial activity of the leaf extracts of *G. sylvestre* as recorded in present study might therefore be attributed to the presence of phytochemicals like flavonoids, terpenoids, amino acids, glycosides, tannins, amino acids and carbohydrates.

CONCLUSIONS

Fresh *Aloe vera* juice can be stored for a period of two weeks at room temperature without much loss in quality and microbial contamination, by the addition of alcoholic extract of *Gymnema sylvestre*.

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